osr1 and *hand2* Act in Opposition to Regulate Formation of Kidney and Vessel Lineages

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Genetic regulation of intermediate mesoderm dimensions and boundary formation is poorly understood

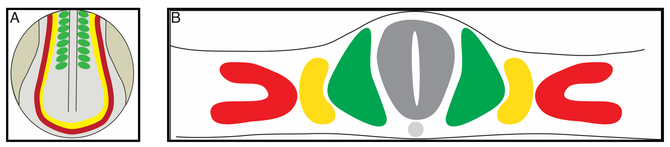

Figure 1. Dorsal view (A) and cross-section (B) of the posterior of a vertebrate embryo. The kidneys arise from the intermediate mesoderm (IM), which lies between the paraxial mesoderm and lateral plate mesoderm (LPM), which gives rise to blood and vessels. Transcription factors, such as Osr1, WT1, Pax2, Pax8, Lim1, and Sim1, are required for IM development. How the dimensions of the IM are determined and how the IM is distinguished from neighboring territories, however, are largely unknown.

hand2 and *osr1* act in opposing, parallel pathways to regulate kidney development

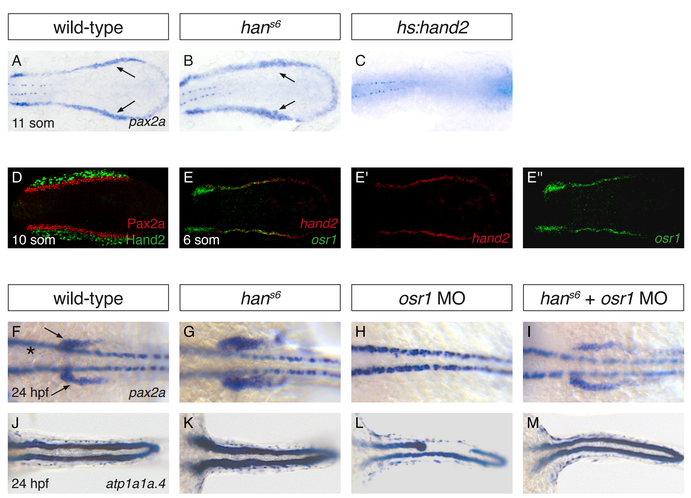

Figure 2. Our initial interest in investigating IM development came from studies of the role of *hand2*, which encodes a bHLH transcription factor, in the development of the zebrafish embryonic kidney, the pronephron (Perens et al., 2016). (A-C) *hand2* inhibits IM formation. Compared to wild-type embryos (A), *pax2a* expression in the IM (arrows) is widened in *hand2* mutants (B) and absent in *hand2*-overexpressing embryos (*hs:hand2*) (C). (D) Hand2 is expressed laterally adjacent to the IM, labeled by Pax2a. (E) Additionally, we found that *osr1*, which encodes a zinc-finger transcription factor well known for its requirement in early kidney development, is expressed in the same lateral territory as *hand2*. (F-I) *osr1* and *hand2* act in parallel, anatagonistic pathways during pronephron development. Compared to wild-type (F), *pax2a* expression in the glomerular precursors (arrows) is expanded in *hand2* mutants (G), absent or reduced in *osr1* morphants (H), and relatively normal in *hand2* mutant + *osr1* MO embryos (I). Expression in overlying spinal neurons (F, asterisk) is unaffected. (J-M) Compared to wild-type (J), *atp1a1a.4* expression in the pronephric tubules is wide in *hand2* mutants (K), while many *osr1* morphants (L) have tubules with shortened anterior expression or segmental losses. Most *hand2* mutant + *osr1* MO embryos (M) resemble wild-type. Dorsal views, anterior to the left.

osr1 is required for intermediate mesoderm and pronephron development

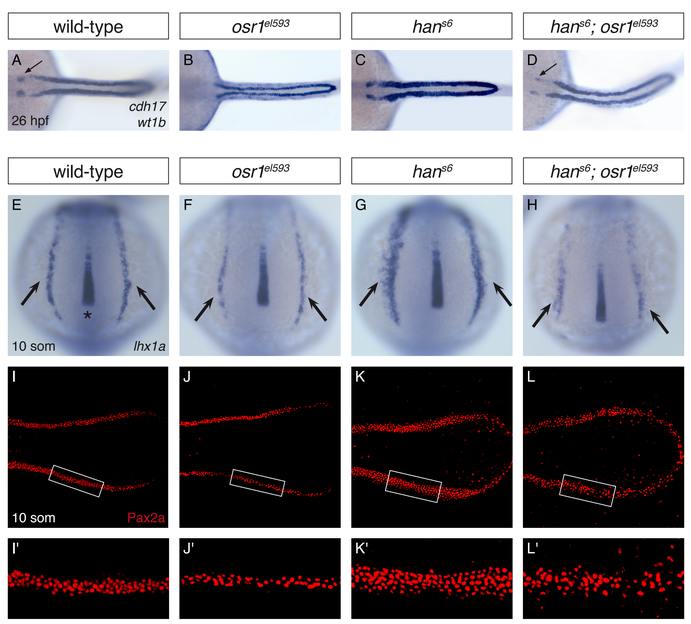

Figure 3. *osr1* (also known as *Odd1*) is expressed in kidney and vascular progenitors in amniotes (James et al., 2006, Mugford et al., 2008), and *Odd1* mutant mice fail to form metanephric kidneys (Jiang et al., 2005). However, the roles of *osr1* in IM and vascular progenitor development are poorly understood. To further investigate the function of *osr1*, we are utilizing a mutation in the zebrafish *osr1* gene; this novel allele is a 7 bp deletion generated using TALEN-mediated genome editing. (A-D) As in *osr1* morphants, formation of the pronephric glomerulus (marked by *wt1b*, arrows) and tubule (marked by *cdh17*) are disrupted in *osr1* mutants. Similarly, each of these *osr1* pronephric defects is partially suppressed by *hand2* (D; arrow in D indicates presence of *wt1b*+ glomerular cells). (E-L) While *osr1* is known to play a vital role in kidney development, the role of *osr1* in IM development is poorly understood. We find that *osr1* mutants exhibit reduced IM differentiation: expression of *lhx1a* (E-H) and Pax2a (I-L) in the IM (arrows) is decreased in *osr1* mutants (F, J) compared to wild-type (E, I). Furthermore, while the IM is increased in *hand2* mutants (G, K), this defect was partially suppressed by the *osr1* mutation (H, L). (E-H) *lhx1a* expression in the notochord (asterisk) was unaffected. (I'–L') Magnification of 250 um long regions from (I-L) used for quantification of the number of Pax2a+ cells. Compared to wild-type (94.2 ± 15.0 Pax2a+ cells/250 um; n=34), there were significantly fewer IM cells in *osr1* mutants (71.7 ± 20.7; n=24; p <0.0001), but comparable numbers of cells in *hand2*; *osr1* double mutants (115 ± 20.9; n=10; p=0.0011). Dorsal views, anterior to the left (A-D, I-L) or anterior to the top (E-H).

osr1 suppresses emergence of lateral vessel progenitors

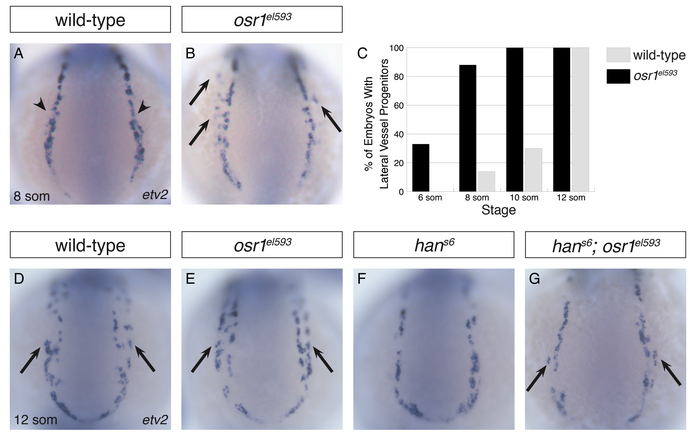

Figure 4. Previously, we found that *hand2* is required for the formation of a subset of lateral vessel progenitors within the posterior mesoderm (Perens et al, 2016). Considering the interaction between *hand2* and *osr1* in IM development, we sought to determine the role of *osr1* in the formation of these vessel progenitors. In the posterior mesoderm, *etv2* is first expressed in bilateral vascular progenitors located medial to the IM (arrowheads, A). During somitogenesis, the lateral vessel progenitors emerge most often between the 10-12 somite stages (C; arrows, D). In *osr1* mutants, the lateral vessel progenitors emerge prematurely at the 8 somite stage (arrows, B; C). (C) While most wild-type animals form *etv2*+ lateral vessel progenitors between the 10-12 somite stages, most *osr1* mutants form these cells between the 6-8 somite stages. (D-G) Additionally, while the lateral vessel progenitors fail to form in *hand2* mutants (Perens et al, 2016; F), some progenitors form in *hand2*; *osr1* double mutants (arrows, G). Thus, as with the IM phenotype, *osr1* partially suppresses the *hand2* mutant lateral venous progenitor phenotype. Dorsal views, anterior to the top.

Expression of *osr1* in the posterior lateral mesoderm decreases during somitogenesis

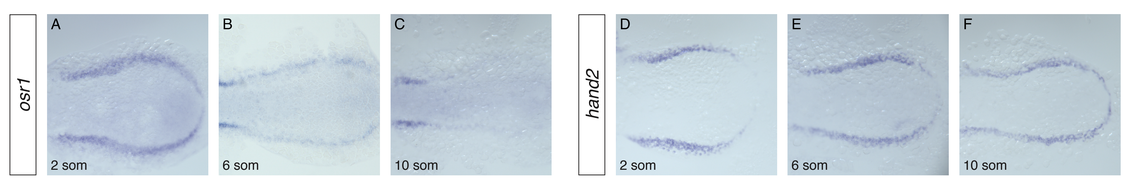

Figure 5. Considering the role of *osr1* in regulating the timing of lateral vessel progenitor emergence, we hypothesized that *osr1* expression dynamics may correlate with lateral vessel progenitor development. (A-C) While *osr1* is broadly expressed in the posterior lateral mesoderm at the beginning of somitogenesis (A), expression decreases signficantly at the 6 (B) and 10 (C) somite stages. (D-F) Conversely, *hand2*, which we previously found to be co-expressed with *osr1* in the posterior lateral mesoderm (Fig. 2 and Perens et al., 2016), remains strongly expressed throughout early somitogeneis. Thus, *osr1* expression dynamics may couple *osr1* function with lateral vessel progenitor emergence. Dorsal views, anterior to the left.

osr1 is sufficient to suppress formation of lateral vessel progenitors and to promote formation of intermediate mesoderm

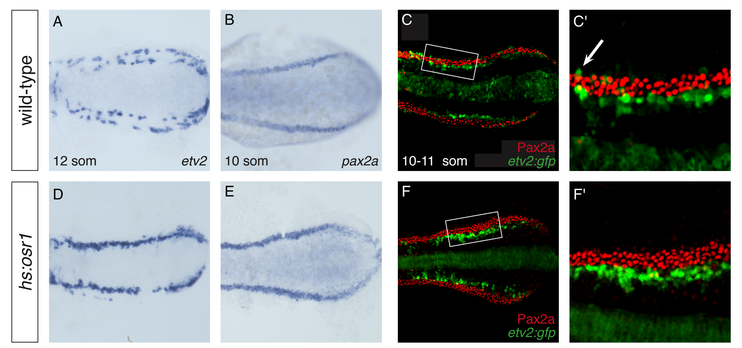

Figure 6. Because decreased *osr1* expression correlated temporally with the emergence of the lateral vessel progenitors, we sought to determine whether increased *osr1* expression could suppress their formation. In contrast to wild-type embryos (A), which form two bilateral stripes of *etv2*-expressing vessel progenitors, embryos overexpressing *osr1* (*hs:osr1*) (D) only form one stripe of *etv2* expression. (B, E) Furthermore, analysis of the IM, marked by *pax2a* expression, demonstrated a subtle, but consistent increase in *hs:osr1* embryos. (C,F) To further interrogate these phenotypes, we examined embryos co-stained for Pax2a and *etv2:gfp*. Notably, the single stripe of *etv2* expression in *hs:osr1* embryos is located medial to Pax2a expression, consistent with *osr1* overexpression inhibiting lateral vessel progenitor formation. Furthermore, compared to wild-type (93.9 ± 10.8 Pax2a+ cells/250 um; n=10), there was a modest increase in IM cells in *hs:osr1* embryos (111.4 ± 22.3; n=40; p=0.0018). (C',F') Magnification of 250 um long regions from (C,F) used for quantification of the number of Pax2a+ cells. Dorsal views, anterior to the left.

Conclusions and Future Directions

- *osr1* is required for pronephron and IM formation.
- *osr1* regulates the timing of lateral vessel progenitor emergence.
- Levels of *osr1* expression may couple developmental timing and cell fate dynamics within the posterior mesoderm.

- Do the intermediate mesoderm, vessel progenitor cells and/or *osr1*-expressing cells share a common progenitor?
- Which genes do *osr1* and *hand2* regulate to coordinate specification of the IM and the vessel progenitors?

Acknowledgements

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